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PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Peter	Cox	44 Newton Road, Cambridge
Alistair	Dixon	108 Gwydir Street, Cambridge, CB1, 2LL
Anthony P.	Jackson	6 Leys Avenue, Cambridge, CB4 2AW
Kevin	Morgan	30 Toft Lane, Great Wilbraham, Cambridge, CB1 5JH

☐ *Additional inventors are being named on page 2 attached hereto*

TITLE OF THE INVENTION (280 characters max)

A NOVEL BETA SUB-UNIT FROM A VOLTAGE-GATED SODIUM CHANNEL, NUCLEIC ACIDS ENCODING THEM AND THERAPEUTIC OR DIAGNOSTIC USES THEREOF.

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR

☒ Firm *or* Individual Name	Charles W. Ashbrook				
Address	Warner-Lambert Company				
Address	2800 Plymouth Road				
City	Ann Arbor, Michigan	State	Michigan	ZIP	48105
Country	United States	Telephone	(734) 622-5215	Fax	(734) 622-1553

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification	*Number of Pages*	44		
☒ Drawing(s)	*Number of Sheets*	3	☒ Other (specify)	49 Claims on 5 Pgs.; Abstract on 1 Page

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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Respectfully submitted,

SIGNATURE Charles W. Ashbrook DATE April 15, 1999

TYPED or PRINTED NAME Charles W. Ashbrook REGISTRATION NO. *(if appropriate)* 27,610

TELEPHONE (734) 622-5215

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A novel beta sub-unit from a voltage-gated sodium channel, nucleic acids encoding them and therapeutic or diagnostic uses thereof.

PARKE DAVIS

TITLE OF THE INVENTION

A novel family of beta sub-unit proteins from a voltage-gat d sodium channel, nucleic acids encoding them and therapeutic or diagnostic uses thereof.

FIELD OF THE INVENTION

The present invention relates to a novel family of beta sub-unit proteins from a voltage- gated sodium channel, and particularly the human and the rat beta sub-units which have been collectively identified as β3, in view their close structural relationship.

The invention also deals with the use of the β3 sub-unit polypeptide or a fragment thereof as well as of the nucleic acids encoding same for therapeutic and diagnostic purposes.

BACKGROUND OF THE INVENTION

Sodium channels play a central role in physiology. They transmit depolarising impulses rapidly throughout cells and cell networks, thereby enabling co-ordination of higher processes from cognition to locomotion. The ion permeability and voltage sensing is primarily determined by the alpha sub-unit of the sodium channel complex as this forms the pore. There are at least two major classes and at least eight genes encoding sodium channels.

Voltage-dependent Na^+ channels have long been recognized as targets for anti-arrhythmic and local anaesthetic drugs. Since the mid-1980s , Na^+ channels have become widely accepted as the primary target of anticonvulsants with pharmacological profiles similar to those of phenytoin, carbamazepine, and lamotrigine.

Alteration of ion channel function is an important pathophysiological mechanism of various familial muscle diseases. Na^+ channel mutations underlie the aberrant excitability characteristic of some skeletal muscle myotonias and paralysis, as well as chromosome 3-linked long-QT syndrome, an inherited cardiac arrhythmia. In general, these mutations disable inactivation of the Na^+ channel, producing either repetitive action potential firing (myotonia) or electrical silence (flaccid paralysis) in skeletal

muscles. A similar defect in the cardiac Na^+ channel produces action potential prolongation and a predisposition to repetitive electrical activity in the heart leading to polymorphic ventricular tachycardia.

Additional determinants of sodium channel function are the presence or absence of auxiliary β1 and 2 sub-units. These are important modulators of Na^+ channel function. Biochemical studies first revealed the existence of two distinct sub-units (β1 and β2) associated with the brain Na^+ channel. Antibodies directed to the α or β sub-unit appeared to immunoprecipitate the entire brain Na^+ channel complex with a sub-unit stoichiometry of 1α: 1β1: 1β2. The β1 sub-unit is non-covalently associated, while β2 is linked by a disulphide bond to the α sub-unit. The β1 and β2 sub-units have been cloned and the deduced primary structures indicate that they are unrelated proteins of molecular weights of 23 and 21 kDa, respectively. The predicted transmembrane topology of the sub-units is similar: each contains a small carboxy-terminal cytoplasmic domain, a single membrane-spanning segment, and a large amino-terminal extracellular domain with several consensus sites for N-linked glycosylation.

Expression of β2 with neuronal sub-units in Xenopus oocytes increased the current amplitude, modulated gating and increased the membrane capacitance. Co-expression of β1 sub-units with either neuronal or skeletal muscle sub-units in oocytes also produced clear-cut effects on channel function. The current density increased, activation and inactivation gating were accelerated, and the steady-state inactivation curves were shifted in the hyperpolarizing direction. The mRNA encoding the β1 sub-unit appears to be widely expressed and clearly forms an important component of neuronal and skeletal muscle Na^+ channels. It has recently been established that β 1 sub-units modify the interactions of neurotoxins and local anaesthetics with the rat brain α sodium channel.

Until recently there was no known linkage of a phenotype with the β sub-units. However, a mutation in the β 1 sub-unit gene SCNIB has been shown to be associated with Febrile seizures and generalised epilepsy.

All the sub-units of the Na^+ channel are modified by glycosylation. The β1 , β2 and brain and muscle sub-units are heavily glycosylated, with up to 40% of the mass being carbohydrate. In contrast, the cardiac sub-unit contains only 5% of sugar by weight. Sialic acid is a prominent component of

the N-linked carbohydrate of the Na^+ channel. The addition of such a highly charged carbohydrate has predictable effects on the voltage dependence of gating through alteration of the surface charge of the channel protein. Neuraminidase treatment to remove sialic acid from expressed skeletal muscle channels produces a depolarizing shift of steady-state inactivation. It has also been shown that co-translational glycosylation is essential for the maintenance of cell surface expression of the Na^+ channel in neurones and Schwann cells. Inhibition of glycosylation by tunicamycin reversibly decreases the number of STX binding sites on neuroblastoma cells. Tunicamysin also inhibits palpitation, sulphation and disulphide attachment of the β2 sub-unit, preventing the assembly of functional Na^+ channels.

SUMMARY OF THE INVENTION

The invention relates to a purified or isolated nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel or a sequence complementary thereto.

The invention also concerns a β3 sub-unit polypeptide or a peptide fragment thereof as well as antibodies specifically directed against such β3 sub-unit polypeptide or peptide fragment.

Oligonucleotide probes or primers specifically hybridizing to a nucleic acid encoding a β3 sub-unit or to a sequence complementary thereof are also part of the invention as well as DNA amplification and detection methods using said primers and probes.

A further object of the invention consists of recombinant vectors comprising any of the nucleic acid sequence described herein, and in particular recombinant vectors comprising a nucleic acid sequence encoding a β3 sub-unit of the invention, the invention also encompasses host cells and transgenic non-human mammals comprising said nucleic acid sequences or recombinant vectors.

The present invention is also directed to a method of screening for agonist and antagonist molecules or substances of sodium channels as well as to gene therapy methods involving selective addition or removal of the β3 sub-unit nucleic acid sequence in a genome, particularly via an anti-sense technology.

The invention also pertains to methods for the diagnosis of diseases involving a dysfunction of a voltage-gated sodium channel through analysis, with either the oligonucleotides or antibodies of the invention, of the expression of the β3 sub-unit, more particularly during pain, epilepsy, stroke and ischemia.

The invention will be described hereinafter more in details and will be illustrated by the following figures:

Figure 1: Sequence alignment of the human and rat β3 sub-unit coding sequences.

Upper line: coding sequence of the human β3 sub-unit.

Middle line: coding sequence of the rat β3 sub-unit.

Lower line: consensus sequence containing the nucleotides that are common to both the human and rat β3 sub-units.

Figure 2: Distribution of the sodium channel IIA α and β sub-units in rat brain by in situ hybridization. X-ray autoradiographs of rat brain sagittal sections showing the distribution of rat IIA α (A,B), rat β_1 C,D) and rat β_3 (E,F) mRNA transcripts as revealed by in situ hybridisation with specific olignucleotide probes. Slides were exposed to X-ray film for 10 days. Dark areas indicate high expression levels. Cb, cerebellum; Ctx, cortex; CP, caudate putamen.

Figure 3: Na^+ current curves in oocytes expressing either the IIA α sub-unit alone, or IIA α and β1 or β3 sub-units. Inward Na^+ currents were evoked by applying 5 mV depolarizing pulses from a holding potential of -100 mV, from -80 mV to +30 mV. **a.** Na^+ currents recorded from oocytes expressing IIA α subunit alone. Inactivation at –10 mV was best-fitted with a double exponential function, where τ_1 = 2 ± 0.3 ms and τ_2 = 12.7 ± 2.4 ms (n = 4). **b.** Na^+ currents recorded from oocytes coexpressing IIA α and β_1 subunits. Inactivation was best-fitted with a double exponential function, where τ_1 = 1.3 ± 0.3 ms and τ_2 = 22.7 ± 7.7 ms at -10 mV (n = 4). **c.** Na^+

currents recorded from oocytes coexpressing IIA_2 α and the β_3 subunits. Inactivation was best-fitted with a double exponential function, where τ_1 = 1 ± 0.1 ms and τ_2 = 23.8 ± 6.3 ms at -10 mV (n = 4).

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found a novel family of beta sub-unit proteins that cooperate with at least one α sub-unit of voltage-gated sodium channels to form an active sodium channel. This novel beta sub-unit family has been termed β3 and can be identified as such through common structural sequence features, such as a high homology within the sequences that will be described hereafter.

The inventors have found novel nucleic acid sequences encoding a β3 sub-unit from a voltage-gated sodium channel. They have shown that this β3 sub-unit was biologically functional and that co-expression of the β3 sub-unit with an $\alpha2$ sub-unit from a voltage-gated sodium channel significantly increases the rate of inactivation of the channel, as compared with the expression of the $\alpha2$ sub-unit alone. Moreover, co-expression of the β3 sub-unit of the invention with an $\alpha2$ sub-unit increases the rate of recovery from inactivation of the sodium channel as compared with the expression of the $\alpha2$ sub-unit alone.

The inventors have thus demonstrated that the β3 sub-unit of the invention is involved in the regulation of the sodium currents induced by the voltage-gated sodium channels. They have also determined that the β3 sub-units of the invention may be valuable targets for drugs capable of up regulating or down regulating the activity of voltage-gated sodium channels, in particular drugs designed for preventing or treating pain, epilepsy (typically febrile seizures and generalized epilepsy), stroke and ischemia. In another aspect of the present invention, the nucleic acids encoding the β3 sub-unit may be used to design polynucleotides that can interfere with the functional expression of the β3 sub-unit both *in vitro* and *in vivo*.

Consequently, a first object of the present invention consists of a purified or isolated nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, or a sequence complementary thereto.

By a subtractive PCR technique using total mRNA from wild type PC12 and variant PC12 cell lines, the inventors have isolated a cDNA encoding the rat β3 sub-unit.

From the rat β3 sub-unit cDNA sequence information, the inventors have also isolated and cloned the human cDNA encoding the human β3 sub-unit from a voltage-gated sodium channel.

As shown in figure 1, the coding sequences (ORF) of the rat and human β sub-units are highly homologous, with only 70 non identical nucleotides out of a total length of 648 nucleotides (> 89% nucleotide identity between the two coding sequences).

Consequently, the invention also deals with a purified or isolated nucleic acid which encodes a β3 sub-unit from a voltage-gated sodium channel present in a mammal, preferably a rat brain, or a sequence complementary thereto.

The invention also concerns a purified or isolated nucleic acid which encodes a β3 sub-unit from a voltage-gated sodium channel present in the human brain, or a sequence complementary thereto.

Another object of the invention consists of a purified or isolated nucleic acid encoding a polypeptide having at least 80%, preferably 90%, more preferably 95%, and most preferably 98% aminoacid identity with the rat polypeptide of the aminoacid sequence of SEQ ID N°8 or with a peptide fragment thereof, or a sequence complementary thereto.

The invention further concerns a purified or isolated nucleic acid encoding a polypeptide having at least 80%, preferably 90%, more preferably 95%, and most preferably 98% aminoacid identity with the human polypeptide of the aminoacid sequence of SEQ ID N°9 or with a peptide fragment thereof or a sequence complementary thereto.

Polypeptides having aminoacid identity with the β3 sub-unit of the invention encompass polypeptides:

(a) that are structurally related to the β3 sub-unit of any one of the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9, due to the high sequence identity between the aminoacid sequences; or

(b) that are biologically related to the polypeptides of any one of the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9, either because these homologous polypeptides are recognized by antibodies specifically directed

against the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9 and/or because these homologous polypeptides have the same biological activity as the polypeptides of the aminoacid sequences of SEQ ID N°8 and/or SEQ ID N°9, such as for example the capacity of increasing significantly th rate of inactivation of the sodium channel when co-expressed with an α2 sub-unit, as compared with the capacity of the α2 sub-unit alone, or the capacity of increasing, in the same conditions, the rate of recovery from inactivation of the sodium channel. Suitable assays to determine the biological activity of a sodium channel including a β3 sub-unit of the invention are, for example, described in Example 4 of the present specification.

A further object of the invention consists of a purified or isolated nucleic acid having at least 90%, preferably 95%, more preferably 98%, and most preferably 99% nucleotide identity with the nucleotides sequence of SEQ ID N°1, or a sequence complementary thereto.

The invention also deals with a purified or isolated nucleic acid comprising a sequence encoding the Open Reading Frame (ORF) of a β3 sub-unit from a voltage-gated channel present in the rat brain, such sequence having at least 90%, preferably 95%, more preferably 98% and most preferably 99% with the polynucleotide beginning at the nucleotide located in position 363 and ending at the nucleotide located in position 1010 of the nucleotide sequence of SEQ ID N°1.

The invention relates also to a purified or isolated nucleic acid having at least 90%, preferably 95%, more preferably 98%, and most preferably 99% nucleotide identity with the nucleotide sequence of SEQ ID N°2, or a sequence complementary thereto.

The invention is also directed to a purified or isolated nucleic acid comprising a sequence encoding the Open Reading Frame (ORF) of a β3 sub-unit from a voltage-gated channel present in the human brain, such sequence having at least 90%, preferably 95%, more preferably 98% and most preferably 99% with the polynucleotide beginning at the nucleotide located in position 376 and ending at the nucleotide located in position 1023 of the nucleotide sequence of SEQ ID N°2.

The term " isolated " requires that the material be removed from its original environment (e.g. the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or a peptide present in a

living animal is not isolated, but the same polynucleotide or peptide, separated from some or all of the coexisting materials in the natural system, is isolated.

Such polynucleotide can be part of a vector and/or such polynucleotide or peptide can be part of a composition, and still be isolated in that the vector or composition is not a part of its natural environment.

The term " purified " does not require absolute purity; rather , it is intended as a relative definition.

Purification of starting materials or natural materials to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

Throughout the present specification, the expression " nucleotide sequence " may be employed to designate indifferently a polynucleotide or a nucleic acid. More precisely, the expression " nucleotide sequence " encompasses the nucleic material itself and is not restricted to the sequence information (i.e. the succession of letters chosen among the four base letters) that biochemically characterizes a specific DNA or RNA molecule.

As used interchangeably herein, the term " oligonucleotides ", " nucleic acids " and " polynucleotides " include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form.

Further to its general meaning understood by the one skilled in the art, the term " nucleotide " is also used herein to encompass modified nucleotides which comprise at least one of the following modifications: (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar. For examples of analogous linking groups, purines, pyrimidines, and sugars, see for example PCT publication N°WO 95/04064.

The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, or a combination thereof as well as through any purification methods known in the art.

The invention also encompasses polynucleotide fragments of a nucleic acid encoding a β3 sub-unit of a voltage-gated sodium channel as described herein, that may be useful either to express a peptide fragment, preferably a biologically active peptide fragment, of this β3 sub-unit, as nucleic acid primers or probes for amplification or detection purposes, or as

antisense nucleotides able to regulate the expression of the corresponding gene.

Consequently, the present invention also concerns a purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel described herein, preferably at least 10 consecutive nucleotides of any one of the nucleotide sequences of SEQ ID N°1 or 2, or a sequence complementary thereto.

The nucleic acids described above consist of a contiguous span which ranges in length from 10, 12, 15, 18 or 20 to 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides, or be specified as being 10, 12, 15, 18, 20, 25, 35, 40, 50, 100, 200, 250, 500 or 1000 nucleotides in length.

These nucleic acids are useful as probes in order to detect the presence of at least a copy of a nucleotide sequence encoding a β3 sub-unit from a voltage-gated sodium channel, more particularly the presence of at least a copy of a nucleotide sequence of SEQ ID N°1 or SEQ ID N°2 or a sequence complementary thereto or a fragment or a variant thereof in a sample.

The nucleic acid probes of the invention may also be used for the analysis of the expression levels and patterns of the β3 sub-unit, such as described in the PCT Application N°WO 97/05 277, the entire contents of which is herein incorporated by reference.

Quantitative analysis of the β3 sub-unit expression may also be performed using assays, i.e. a substrate on which has been bound a plurality of nucleic acid probes according to the invention, these probes being either randomly distributed on the substrate or arranged following a one dimensional, two dimensional or multidimensional arrangement. Such assays may additionally comprise nucleic acid probes that do not hybridize with aβ3 sub-unit DNA or RNA, such as for example probes specific for α2, β1 or β2 sodium channel sub-unit RNA or DNA sequences. Suitable techniques are, for example, those described by Schena et al (1995; 1996), and also by Sosnowsky et al., (1997), the disclosures of which are herein incorporated by reference.

The invention further deals with a purified or isolated nucleic acid that hybridizes, under stringent hybridization conditions, with a nucleic acid

encoding a β3 sub-unit from a voltage-gated sodium channel, or a sequence complementary thereto.

As an illustrative embodiment, stringent hybridization conditions can bedefined as follows:

The hybridization step is conducted at 65°C in the presence of 6 x SSC buffer, 5 x Denhardt's solution, 0.5 % SDS and 100µg/ml of salmon sperm DNA.

The hybridization step is followed by four washing steps:

• two washings during 5 minutes, preferably at 65°C in a 2 x SSC and 0.1% SDS buffer;

• one washing during 30 minutes, preferably at 65°C in a 2 x SSC and 0.1% SDS buffer;

• one washing during 10 minutes, preferably at 35°C in a 0.1 x SSC and 0.1% SDS buffer,

it being understood that the hybridization conditions defined above are suitable for nucleic acids of approximately twenty nucleotides in length and that these conditions may be also adapted for shorter or longer nucleic acids, according to techniques well known in the art, for example those described by Sambrook et al. (1989).

The appropriate length for probes under a particular set of assay conditions may be empirically determined by the one skilled in the art. The probes can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis by a method such as the phosphodiester method of Narang et al.(1979), the phosphodiester method of Brown et al., (1979), the diethylphosphoramidite method of Beaucage et al. (1981) and the solid support method described in the application N°EP-0 707 792. The disclosures of all these documents are incorporated herein by reference.

Any of the nucleic acids of the present invention can be labelled, if desired, by incorporating a label detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

For example, useful labels include radio-active substances (^{32}P, ^{35}S, ^{3}H, ^{125}I), fluorescent dyes (5-bromodesoxyuridin, fluorecein, acetylaminofluoren, digoxygenin) or biotin. Examples of non-radioactive

labelling of nucleic acid fragments are described in French Patent N°FR-78 10975 or by Urdea et al. (1988) or Sanchez-Pescador et al. (1988).

Advantageously, the probes according to the present invention may have structure and characteristics such that they allow signal amplification, such structural characteristics being, for example, branched DNA probes as those described by Urdea et al. (1991).

Any of the nucleic acid probes of the invention can be conveniently immobilized on a solid support. Solid supports are known those skilled in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitro-cellulose strips, membranes, microparticules such as latex particles, sheep red blood cells, duracytes and others.

The nucleic acids of the invention and particularly the nucleotide probes described above can thus be attached to or immobilized on a solid support individually or in groups of at least 2, 5, 8, 10, 12, 15, 20 or 25 distinct nucleic acids of the invention to a single solid support.

In a specific embodiment of a support on which nucleic acid probes of the invention are immobilized, such a support may also contain other immobilized probes, preferably probes that hybridize specifically with a nucleic acid encoding a sub-unit from a voltage-gated sodium channel, or a variant thereof, or a sequence complementary thereto, and more preferably with a nucleic acid encoding an α sub-unit, most preferably an α_2 sub-unit of a voltage-gated sodium channel.

The invention also encompasses nucleic acid probes comprising a nucleotide sequence included in any one of the sequences of SEQ ID N°1 and 2 wherein at least one nucleotide substitution has been made in order to create a mismatch between this probe and the complementary nucleotide sequence included in any one of SEQ ID N°1 and SEQ ID N°2 to be detected. Under suitable hybridization conditions, these probes will not hybridize anymore with anyone of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2 or a fragment thereof or a sequence complementary thereto, but will hybridize only with nucleotide sequences which are exactly complementary to the polynucleotide comprised in these probes.

This specific embodiment of the nucleic acid probes of the invention may allow the detection of nucleotide polymorphisms within a nucleic acid sequence encoding a $\beta 3$ sub-unit of voltage-gated sodium channel, more

specifically in a nucleic acid encoding a β3 sub-unit voltage-gated sodium channel from human or rat, and more preferably a nucleic acid of any one of the sequences SEQ ID N°1 and SEQ ID N°2, or a sequence complementary thereto.

Such probes can allow the one skilled in the art to detect mutations occurring in a nucleic acid encoding a β3 sub-unit of the invention, more preferably a nucleic acid encoding a β3 sub-unit from rat of human, and most preferably a nucleic acid or any one of SEQ ID N°1 and SEQ ID N°2.

The invention also deals with a method for detecting the presence of a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, a fragment or a variant thereof or a complementary sequence thereto in a sample, said method comprising the following steps:

(a) bringing into contact a nucleic acid probe or a plurality of nucleic acid probes of the invention which can hybridize with a nucleotide sequence included in a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, or a fragment a variant thereof or a complementary sequence thereto, and a sample to be assayed;

(b) detecting the hybrid complex formed between the probe or the plurality of probes and a nucleic acid in the sample.

In a first preferred embodiment, the nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel to be detected is preferably a rat or human β3 sub-unit, and more preferably a nucleic acid selected from the group consisting of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2.

In a second preferred embodiment of this detection method, thenucleic acid probe or the plurality of nucleic acid probes are labelled with a detectable molecule.

In a third preferred embodiment of the method, the nucleic acid probe or the plurality of nucleic acid probes are immobilized on a substrate.

In a fourth preferred embodiment of the method, the nucleic acid contained in the sample is made available to hybridization before step (a), by any conventional procedure well known from the one skilled in the art.

The invention further concerns a kit for detecting the presence of a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, a

fragment or a variant thereof or a complementary sequence thereto in a sample, wherein said kit comprises:

(a) a nucleic acid probe or a plurality of a nucleic acid probes as described above;

(b) optionally, a reagent necessary for performing the hybridization reaction.

In a first preferred embodiment of the detection kit, the nucleic acid to be detected encodes a human or rat β3 sub-unit, and consists preferably of any one of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2, a fragment or a variant thereof, or a sequence complementary thereto.

In a second preferred embodiment of the detection kit, the nucleic acid probe or the plurality of nucleic acid probes are labelled with a detectable molecule.

In a third preferred embodiment of the detection kit, the nucleic acid probe or the plurality of nucleic acid probes are immobilized on a substrate.

The invention is also directed to a polynucleotide primer hybridizing, under the stringent hybridization conditions described herein, with a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel of the invention, preferably a rat or a human β3 sub-unit, and more preferably a nucleotide sequence selected from the group consisting of SEQ ID N°1 and SEQ ID N°2.

As an illustrative example, primers according to the present invention may comprise, or may consist of a polynucleotide selected from the group consisting of the nucleotide sequences of SEQ ID N°4 and SEQ ID N°5. The use of a pair of primers respectively comprising, or consisting of the nucleotide sequences of SEQ ID N°4 and SEQ ID N°5 allows the one skilled in the art to amplify the whole nucleic acid sequences encoding either the human β3 sub-unit or the rat β3 sub-unit of the invention.

In a specific embodiment of a primer according to the invention, such a primer may comprise a 3' end nucleotide which is not exactly complementary to a target sequence included in anyone of the nucleotide sequences of SEQ ID N°1 or SEQ ID N°2, or a sequence complementary thereto.

According to this specific embodiment of a primer according to the invention, such a primer comprises a 3' end nucleotide chosen in such a way

as to allow hybridization of the primer, and thus the possibility of further elongation, only when a given variant of a β3 sub-unit of the invention is present in the sample containing the target sequence to be amplified, this variant β3 sub-unit nucleic acid sequence corresponding to a genome polymorphism. Particularly, preferred primers encompassed in this specific embodiment will then exclusively hybridize with a given variant of a β3 sub-unit of the invention, and more preferably with a β3 sub-unit of the invention for which a linkage with a detectable phenotype, caused by a disfunction in a voltage-gated sodium channel, and more preferably with pain, epilepsy, stroke and ischemia, has been shown.

Another object of the invention consists of a method for the amplification of a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, said method comprising the steps of:

(a) contacting a test sample suspected of containing the target β3 sub-unit nucleic acid, a fragment or a variant thereof, or a sequence complementary thereto, with an amplification reaction reagent comprising a pair of amplification primers located on either side of the β3 sub-unit nucleic acid region to be amplified, and

(b) optionally, detecting the amplification products.

In a first preferred embodiment of the above method, the nucleic acid encodes a human or rat β3 sub-unit, and more preferably a β3 sub-unit of any one of the amino acid sequences of SEQ ID N°8 or SEQ ID N°9.

In a second preferred embodiment of the above method, the primers comprise, or consist of, any one of the nucleotide sequences of SEQ ID N°4 and SEQ ID N°5.

In a third preferred embodiment of the above amplification method, the amplification product is detected by hybridization with a labelled probe having a sequence which is complementary to the amplified region.

The invention also concerns a kit for the amplification of a nucleic acid encoding a β3 sub-unit from voltage-gated sodium channel, a fragment or a variant thereof, or a complementary sequence thereto in a test sample, wherein said kit comprises:

(a) a pair of oligonucleotide primers located on either side of the β3 sub-unit nucleic acid region to be amplified;

(b) optionally, the reagents necessary for performing the amplification reaction.

In a first preferred embodiment of the kit described above, the nucleic acid encodes a human or a rat β3 sub-unit, and more preferably a β3 sub-unit of any one of the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9.

In a second preferred embodiment of the above amplification kit, the amplification product is detected by hybridization with a labelled probe having a sequence which is complementary to the amplified region.

In a third embodiment of the above amplification kit, the amplification primers are respectively the nucleotide sequences of SEQ ID N°4 and SEQ ID N°5.

A further object of the invention consists of antisense nucleic acids that inhibit or abolish the expression of the β3 sub-unit gene according to the invention. Preferred methods using antisense nucleic acid according to the present invention are the procedures described by Sczakiel et al. (1995).

Preferably, the antisense nucleic acids are chosen among the polynucleotides of 15-200bp long that are complementary to the 5'end of a nucleic acid encoding a β3 sub-unit protein of the invention, preferably a human or a rat β3 sub-unit, more preferably a β3 sub-unit of any one of the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9, and most preferably a nucleic acid selected from the group consisting of the nucleotides sequences of SEQ ID N°1 and SEQ ID N°2 .

Preferred antisense nucleic acids according to the present invention are complementary to a sequence of the human or rat mRNAs of the β3 sub-unit that contains the translation initiation codon ATG. However, the antisense nucleic acid can also be complementary to a sequence in the 3' or 5' untranslated regions.

The antisense nucleic acids of the invention should have a length and a melting temperature sufficient to permit formation of an intracellular duplex having sufficient stability to inhibit the expression of the desired β3 sub-unit in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green et al. (1986) and Izant and Weintraub (1984), the disclosures of which are incorporated herein by reference.

Alternative suitable antisense strategies are those described by Rossi et al. (1991), in PCT Applications Nos WO 94/23 026, WO 95/04141, WO 92/18 522 and in the European Patent Application N° EP-0 572 287 A2, incorporated herein by reference.

The preferred antisense nucleic acid sequence according to the present invention is the nucleotide sequence of SEQ ID N°3.

For designing antisense nucleic acids according to the present invention, the one skilled in the art may also be guided by the teachings of the publication of Zhiqiang Zhang et al.(1998), the disclosure of which is herein incorporated by reference.

Without wishing to be bound by any particular theory, the inventors believe that one way in which modification of sodium channels including a β3 sub-unnit can alter excitability would be the following mechanism. The decay of the sodium current can be fitted by at least two exponents. The major component of these two currents is significantly shortened by co-expression of the β3 sub-units. This is due to the β3 sub-unit significantly shifting the voltage activation curve in a positive direction allowing more rapid repolarization of the membrane potential. Thus an increase in the number of events or transfer of information down the axon occurs. The high density of sodium channels expressed in neurones allows conduction of action potentials to occur over the entire cell surface. Transient removal of sodium channels from the membrane may afford a reversible method of changing the gain function of a nerve terminal to depolarising input.

Consequently, the inventors believe that inhibition of the expression of the β3 sub-unit of the invention, for example via an antisense strategy, may affect the expression and/or the surface expression of the voltage-gated sodium channel of which the β3 sub-unit is part , and consequently affect the expression and the biological activity of the whole voltage-gated sodium channel. Such a voltage-gated sodium channel inhibition may be useful for preventing or curing diseases like epilepsy, hyperalgesia and cardiovascular diseases.

Additionally, increasing the inactivation of sodium channels will lead to a damping effect on neuronal excitability.

Moreover, it is suggested that the β3 sub-units are tightly bound to the α pore and that trafficking of the complex to the appropriate place requires

the β3 sub-unit. Thus, a modification, preferably an inhibition, of the β3-α complex may be used to increase sequestration to an intracellular site or reduced trafficking of α and β to terminal membrane reagents. This would reduce excitability since current density during depolarisation would be insufficient to maintain propagation of the action potential.

Furthermore, changing the expression of the β3 sub-unit would cause a disruption of sodium channel function in an injured region.

Another object of the invention is the use of the nucleic acids encoding the β3 sub-unit or a biologically active peptide fragment thereof in gene therapy, by insertion of the fully functional gene by a vector delivery system that would result in the repair of a damaged area . In order to affect expression of the nucleic acids encoding a β3 sub-unit protein of the invention, these nucleic acids must be delivered into a cell. This delivery may be accomplished *in vitro,* as in laboratory procedures for transforming cell lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states, particularly disease states related to a dysfunction in the voltage-gated sodium channels, and more particularly disease states such as pain, epilepsy, stroke, ischemia, hyperalgesia and cardiovascular disease.

One mechanism is viral infection where the nucleic acid to be expressed is encapsulated in an infectious viral particle. Several non-viral methods for the transfer of polynucleotides into cultured mammalian cells are also contemplated by the present invention, and include, without being limited to, calcium phosphate precipitation: Graham et al., (1973); Chen et al., (1987), DEAE-dextran Gopal: (1985), electroporation: Tur-Kaspa et al. , (1986); Potter et al., (1984), direct micro-injection: Harland et al., (1985), and DNA-loaded liposomes: Nicolau et al., (1982); Fraley et al. , (1979).

Once the nucleic acid to be expressed has been delivered into the cell, it may be stably integrated into the genome of the recipient cell. This integration may be in the right location and orientation via homologous recombination (gene replacement) or it may be in a random, non specific location. In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or episomes encode sequences sufficient to permit maintenance and replication independent of, or in synchronization with the

host cell cycle. A suitable gene targeting technique is described in Russel (1998), the disclosure of which is herein incorporated by reference.

One specific embodiment of a method for delivering a nucleic acid to the interior of a cell of a vertebrate *in vivo* comprises the step of introducing a preparation comprising a physiologically acceptable carrier and a naked polynucleotide operatively coding for the polypeptide of interest into the interstitial space of a tissue comprising the cell, whereby the naked polynucleotide is taken up into the interior of the cell and has a physiological effect.

Compositions for use *in vitro* and *in vivo* comprising a naked polynucleotide are described in PCT Application N° WO 90/11 092 (Vical Inc.) as well as in the articles of Tacson et al. (1996) and of Huygen et al., (1996), the disclosures of which are herein incorporated by reference.

Another object of the invention consists of a composition for the *in vivo* production of a β3 sub-unit protein or a biologically active peptide fragment thereof. Such a composition may comprise a naked polynucleotide operatively coding for this polypeptide, in solution in a physiologically acceptable carrier, and suitable for introduction into a tissue to cause cells of the tissue to express a functional β3 sub-unit protein or a peptide fragment thereof and thus a functional voltage-gated sodium channel.

The amount of vector to be injected to the desired host organism varies according to the site of injection. As an indicative dose, it will be injected between 0.1 and 100 μg of the vector in an animal body, preferably a mammal body, and preferably a human body.

In another embodiment of a gene therapy method of the invention, the nucleic acid that operatively expresses the β3 sub-unit protein or a biologically active peptide fragment thereof may be introduced in vitro in a host cell, preferably in a host cell previously harvested from the animal to be treated and more preferably a somatic cell such as a muscle cell or a neuronal cell. In a subsequent step, the cells that have been transformed with the nucleic acid encoding the β3 sub-unit protein or its peptide fragment of interest is reintroduced into the animal body in order to deliver the recombinant protein within the body either locally or systemically.

Therefore, the invention is also directed to a composition containing a nucleic acid selected from the group of nucleic acids described therein, in

combination with one or several physiologically acceptable carriers, such as those well known from the one skilled in the art.

The present invention also encompasses a family of recombinant vectors comprising any one of the nucleic acids described herein. Thus, the invention further deals with a recombinant vector comprising a nucleic acid selected from the group consisting of :

(a) a purified or isolated nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, preferably a human or a rat β3 sub-unit, and more preferably a polypeptide having at least 80% amino acid identity with a polypeptide selected from the group consisting of the amino acid sequences of SEQ ID N°8 and SEQ ID N°9, or a sequence complementary thereto;

(b) a purified or isolated nucleic acid having at least 90% nucleotide identity with a polynucleotide selected from the group consisting of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2, or a sequence complementary thereto;

(c) a purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid described in (a) or (b), or a sequence complementary thereto.

In a first preferred embodiment a recombinant vector of the invention is used to amplify the inserted polynucleotide derived from the nucleic acid encoding a β3 sub-unit of the invention in a suitable host cell, this polynucleotide being amplified every time the recombinant vector replicates.

A second preferred embodiment of the recombinant vectors according to the invention consists of expression vectors comprising a nucleic acid encoding a β3 sub-unit of the invention, preferably a nucleic acid encoding a human or a rat β3 sub-unit, and more preferably a nucleic acid encoding a polypeptide selected from the group consisting of the amino acid sequences of SEQ ID N°8 and SEQ ID N°9, and most preferably a nucleic acid selected from the group consisting of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2.

Recombinant expression vectors comprising a nucleic acid encoding the peptide fragments of a β3 sub-unit that are specified in the present specification are also part of the invention .

Within certain embodiments, expression vectors can be employed to express the β3 sub-unit of the invention or a peptide fragment thereof which

can then be purified and for example, be used as a immunogen in order to raise specific antibodies directed against said β3 sub-unit protein or a peptide fragment thereof.

In another embodiment, the expression vectors are used for constructing transgenic animals and also for gene therapy, notably for antisense therapy.

Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in host cells. The regulatory sequences of the expression vectors of the invention are operably linked to the nucleic acid encoding the β3 sub-unit protein of interest or a peptide fragment thereof.

As used herein, the term " operably linked " refers to a linkage of polynucleotide elements in a functional relationship. For instance, a promoter or an enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence.

More precisely, two DNA molecules (such as a polynucleotide containing a promoter region and a polynucleotide encoding a desired polypeptide or polynucleotide) are saidto be " operably linked " if the nature of the linkage between the two polynucleotides does not : (1) result in the introduction of a frame-shift mutation or (2) interfere with the ability of the polynucleotide containing the promoter to direct the transcription of the coding polynucleotide.

Generally, recombinant expression vectors will include origins of replication, selectable markers, permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in an appropriate frame with the translation, initiation and termination sequences, and preferably a leader sequence capable of directing sequences of the translated protein into the periplasmic space or the extra-cellular medium.

In a specific embodiment wherein the vector is adapted for transfecting and expressing desired sequences in mammalian host cells, preferred vectors will comprise an origin of replication from the desired host, a suitable promoter and an enhancer, and also any necessary ribosome

binding sites, polyadenylation site, transcriptional termination sequences, and optionally 5'-flanking non-transcribed sequences.

DNA sequences derived from the SV 40 viral genome, for example SV 40 origin, early promoter, enhancer, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

In another embodiment of a recombinant expression vector of the invention, the 5'-flanking non transcribed sequence may comprise a polynucleotide selected from the group consisting of :

(1) the nucleic acid beginning at the nucleotide in position 1 and ending at the nucleotide in position 362 of the nucleotide sequence of SEQ ID N°1;

(2) the nucleic acid beginning at the nucleotide in position 1 and ending at the nucleotide in position 375 of the nucleotide sequence of SEQ ID N°2.

Additionally, a recombinant expression vector of the invention advantageously also comprises an untranscribed polynucleotide located at the 3'end of the coding sequence (ORF), this 3'-UTR polynucleotide being useful for stabilizing the corresponding mRNA or for increasing the expression rate of the vector insert if this 3'-UTR harbors regulation signal elements such as enhancer sequences.

A preferred 3'-UTR sequence will be selected from the group consisting of the 3'-UTR sequences contained in the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2.

Thus, a further object of the invention consists of a 3'-UTR nucleic acid selected from the group consisting of :

(1) the nucleic acid beginning at the nucleotide in position 1011 and ending at the nucleotide in position 2220 of the nucleotide sequence of SEQ ID N°1;

(2) the nucleic acid beginning at the nucleotide in position 1024 and ending at the nucleotide in position 1261 of the nucleotide sequence of SEQ ID N°2.

Suitable promoter regions used in the expression vectors according to the invention are chosen taking into account the host cell in which the heterologous nucleic acids have to be expressed.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression, or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed.

Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Preferred bacterial promoters are the LacI, LacZ, T3 or T7 bacteriophage RNA polymerase promoters, the lambda PR, PL and trp promoters (a EP-0 036 776), the polyhedrin promoter, or the p10 protein promoter from *baculovirus* (kit Novagen; Smith et al., (1983); O'Reilly et al. (1992).

Preferred selectable marker genes contained in the expression recombinant vectors of the invention for selection of transformed host cells are preferably dehydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for *S. cerevisiae* or tetracycline, rifampicin or ampicillin resistance in *E. coli*, or Levamsaccharase for *Mycobacteria*, this latter marker being a negative selection marker.

Preferred bacterial vectors of the invention are listed hereafter as illustrative but not limitative examples:

pQE70, pQE60, pQE-9 (Quiagen), pD10, phagescript, psiX174, p.Bluescript SK, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene); pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pQE-30 (QIA express).

Preferred bacteriophage recombinant vectors of the invention are P1 bacteriophage vectors such as described by Sternberg N.L. (1992;1994).

A suitable vector for the expression of a β3 sub-unit polypeptide of the invention or a fragment thereof, is a baculovirus vector that can be propagated in insect cells and in insect cell-lines. A specific suitable host vector system is the pVL 1392/1393 *baculovirus* transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC N°CRL 1711) which is derived from *spodoptera frugiperda.*

The recombinant expression vectors from the invention may also be derived from an adenovirus such as those described by Feldman and Steig. (1996) or Ohno et al. (1994).

Another preferred recombinant adenovirus according to this specific embodiment of the present invention is the human adenovirus type two or five (Ad 2 or Ad 5) or an adenovirus of animal origin (French Patent Application n°FR 93 05 954).

Particularly preferred retrovirus as for the preparation or construction of retroviral *in vitro* or *in vivo* gene delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, murine sarcoma virus, and Ross Sarcoma Virus. Other preferred retroviral vectors are those described in Roth et al. (1996), in PCT Application WO 93/25 234, in PCT Application WO 94/06920, and also in Roux et al. (1989), Julan et al.(1992) and Nada et al. (1991).

Yet, another viral vector system that is contemplated by the invention consist in the adeno associated viruses (AAV) such as those described by Flotte et al. (1992), Samulski et al. (1989) and McLaughlin et al. (1996).

Thus, a further object of the invention consists of a recombinant expression vector comprising a nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel or a peptide fragment thereof or a variant thereof, wherein said nucleic acid is operably linked to a promoter sequence.

In a preferred embodiment, this nucleic acid encodes a rat or a human β3 sub-unit, and preferably a β3 sub-unit of any one of the aminoacid sequences of SEQ ID N°8 and SEQ ID N°9, or a variant or a peptide fragment thereof. In a most preferred embodiment, this nucleic acid comprises any one of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2.

Host cells that have been transformed or transfected with one of the nucleic acids described herein, or with one of the recombinant vector, particularly recombinant expression vector, described herein are also part of the present invention.

Are included host cells that are transformed (prokaryotic cells) or are transfected (eukaryotic cells) with a recombinant vector such as one of those described above. Preferred host cells used as recipients for the expression vectors of the invention are the following:

(a) prokaryotic host cells: *Escherichia coli*, strains. (i.e. DH5-α, strain) *Bacillus subtilis, Salmonella typhimurium* and strains from species like *Pseudomonas, Streptomyces* and *Staphylococcus;*

(b) eukaryotic host cells: HeLa cells (ATCC N°CCL2; N°CCL2.1; N°CCL2.2), Cv 1 cells (ATCC N°CCL70), COS cells (ATCC N°CRL 1650; N°CRL 1651), Sf-9 cells (ATCC N°CRL 1711), C127 cells (ATCC N°CRL-1804), 3T3 cells (ATCC N°CRL-6361), CHO cells (ATCC N°CCL-61), human kidney 293 cells (ATCC N° 45504; N°CRL-1573) and BHK (ECACC N°84100 501; N°84111301);

In a specific embodiment of the host cells described above, these host cells have also been transfected or transformed with a polynucleotide or a recombinant vector allowing the expression of another voltage-gated sodium channel sub-unit, preferably a sub-unit of the alpha type, and more preferably a sub-unit of the α2 type, such as described in Example 4. Suitable co-expression procedures are also described in Makielski et al. (1996), and by Qu et al. (1995), the disclosure of which is herein incorporated by reference.

The present invention also concerns a method for producing one of the β3 sub-unit polypeptides described herein and especially a polypeptide selected from the group consisting the aminoacid sequences of SEQ ID N°8 or SEQ ID N°9, wherein said method comprises the steps of:

(a) inserting the nucleic acid encoding the desired β3 sub-unit polypeptide or peptide fragment thereof in an appropriate vector;

(b) culturing, in an appropriate culture medium, a host cell previously transformed or transfected with the recombinant vector of step (a);

(c) harvesting the culture medium thus conditioned or lyse the host cell, for example by sonication or by an osmotic shock;

(d) separating or purifying, from said culture medium, or from the pellet of the resultant host cell lysate, the thus produced β3 sub-unit polypeptide of interest.

In a first preferred embodiment of the above method, the nucleic acid to be inserted in the appropriate vector has previously undergone an amplification reaction, using a pair of primers.

Preferred primers used for such an amplification reaction are the primers of the nucleotide sequences of SEQ ID N°4 and SEQ ID N°5.

In a second preferred embodiment of the above method, the polypeptide thus produced is further characterized, for example by binding onto an immuno-affinity chromatography column on which polyclonal or monoclonal antibodies directed to the β3 sub-unit polypeptide or a peptide fragment thereof have previously been immobilised.

Purification of the recombinant β3 sub-unit proteins according to the present invention or a peptide fragment thereof may be carried out by passage onto a nickel or copper affinity chromatography column.

In another embodiment, the β3 sub-unit polypeptides or peptide fragments thus obtained may be purified, for example, by high performance liquid chromatography, such as reverse phase and/or cationic exchange HPLC, as described by Rougeot et al. (1994).

The reason to prefer this kind of peptide or protein purification is the lack of by-products formed in the elution samples which renders the resultant purified protein or peptide more suitable for therapeutic use.

Another object of the present invention consists of a purified or isolated polypeptide comprising the aminoacid sequence of the β3 sub-unit from a voltage-gated sodium channel, or a peptide fragment or a variant thereof.

In a first embodiment the polypeptide comprises the aminoacid sequence of the β3 sub-unit from a voltage-gated sodium channel present in the rat brain or a peptide fragment or a variant thereof.

In a second preferred embodiment, the polypeptide comprises the aminoacid sequence of the β3 sub-unit or from a voltage-gated sodium channel present in the human brain, or a peptide fragment or variant thereof.

In a third preferred embodiment, the polypeptide comprises an aminoacid sequence having at least 90%, preferably 95%, more preferably 98%, and more preferably 99% amino acid identity with the aminoacid sequence of SEQ ID N°8 or a peptide fragment thereof.

In a fourth embodiment, the polypeptide comprises an aminoacid sequence having at least 90%, preferably 95%, more preferably 98%, and most preferably 99% aminoacid identity with the aminoacid sequence of SEQ ID N°9, or a peptide fragment thereof.

The invention also deals with a purified or isolated polypeptide encoded by a nucleic acid selected from the group consisting of the nucleotide sequences of SEQ ID N°1 and SEQ ID N°2.

Preferred peptide fragments of the β3 sub-unit polypeptide of the invention are those which are involved in covalent or non-covalent interactions with other sub-units of the voltage-gated sodium channel, preferably those involved in covalent or non-covalent interactions with the α sub-unit of the voltage-gated sodium channel. Such polypeptide regions of interactions may be determined by conventional techniques well known to those skilled in the art, such as two hybrid assays as described by Fields and Song, (1989) and also in US Patent N° 5,667,973 as well as in US Patent N°5,283,173 and in Catterall et al. (1998), the technical teachings of these publications being herein incorporated by reference. Other two-hybrid screening assays that may be performed according to the present invention are described by Young et al. (1998), the disclosure of which is also herein incorporated by reference. Other techniques useful to identify biologically relevant peptide fragments or aminoacids involved in the biological activity of the β3 sub-units proteins of the invention are described by Patton et al. (1992), the disclosure of which is herein incorporated by reference.

Other preferred β3 sub-unit peptide fragments are those eliciting the production of antibodies that inhibit or block the normal function of the voltage-gated sodium channel. Such inhibition or blocking of function may be measured by the method described in Example 4 of the present specification.

Preferred peptide fragments such as defined above have at least ten contiguous aminoacids of any one of the amino acid sequences of SEQ ID N°8 or SEQ ID N°9, preferably at least 12 or 15, more preferably at least 20 and most preferably at least 25 consecutive aminoacids of any one of the aminoacid sequences of SEQ ID N°8 or SEQ ID N°9.

The invention also relates to a β3 sub-unit, or a peptide fragment thereof comprising aminoacid changes ranging from 1, 2, 3, 4, 5, 10, 20, 25, 30, 35, 40 substitutions, additions or deletions of one amino acid as regards to the β3 sub-unit polypeptides of anyone of the amino acid sequences of SEQ ID N°8 or SEQ ID N°9.

In the case of an amino acid substitution in the amino acid sequence of a polypeptide according to the invention, one or several consecutive or non-consecutive amino acids are replaced by " equivalent " amino acids. The expression " equivalent " amino acid is used herein to designate any amino acid that may be substituted for one of the amino acids belonging to the native protein structure without decreasing the binding properties of the corresponding peptides to the antibodies raised against the β3 sub-units polypeptides of the amino acid sequence of SEQ ID Nos 8 and 9. In other words, the " equivalent " amino acids are those which allow the generation or the synthesis of a polypeptide with a modified sequence when compared to the amino acid sequence of the native β3 sub-unit protein, said modified polypeptide being able to bind to the antibodies raised against the β3 sub-unit protein of the amino acid sequence of SEQ ID Nos 8 and 9 and/or to induce antibodies recognizing the parent polypeptide.

Alternatively, amino acid changes encompassed are those which will not abolish the biological activity of the resulting modified polypeptide. The biological activity of the modified polypeptide may be assessed, for example, as described in Example 4 of the specification.

These equivalent amino acids may be determined either by their structural homology with the initial amino acids to be replaced, by the similarity of their net charge or of their hydrophobicity, and optionally by the results of the cross-immunogenicity between the parent peptides and their modified counterparts.

The peptides containing one or several " equivalent " amino acids must retain their specificity and affinity properties to the biological targets of the parent protein, as it can be assessed by a ligand binding assay or an ELISA assay.

Examples of amino acids belonging to specific classes include Acidic (Asp, Glu), Basic (Lys, Arg, His), Non-polar (Ala, Val, Leu, Ile, Pro, Met, Phe, Trp) or uncharged Polar (Gly, Seu, Thr, lys, Tyr, Asn, Gln) amino acids.

Preferably, a substitution of an aminoacid in a β3 sub-unit polypeptide of the invention, or in a peptide fragment thereof, consists in the replacement of an aminoacid of a particular class for another aminoacid belonging to the same class.

By an equivalent amino acid according to the present invention is also contemplated the replacement of a residue in the L-form by a residue in the D form or the replacement of a Glutamic acid (E) residue by a Pyro-glutamic acid compound. The synthesis of peptides containing at least one residue in the D-form is, for example, described by Koch (1977).

A specific embodiment of a modified peptide of interest according to the present invention, includes, but is not limited to, a peptide molecule which is resistant to proteolysis. This is a peptide in which the -CONH- peptide bond is modified and replaced by a (CH_2NH) reduced bond, a (NHCO) retro inverso bond, a (CH_2-O) methylene-oxy bond, a (CH_2S) thiomethylene bond, a (CH_2CH_2) carba bond, a (CO-CH_2) cetomethylene bond, a (CH_0H-CH_2) hydroxyethylene bond), a (N-N) bound, a E-alcene bond or also a -CH=CH-bond.

The invention also encompasses a β3 sub-unit polypeptide or a fragment thereof in which at least one peptide bond has been modified as described above.

The polypeptides according to the invention may also be prepared by the conventional methods of chemical synthesis, either in a homogenous solution or in solid phase. As an illustrative embodiment of such chemical polypeptide synthesis techniques, it may be cited the homogenous solution technique described by Houbenweyl (1974) .

The β3 sub-unit polypeptide of interest, or a fragment thereof may thus be prepared by chemical synthesis in liquid or solid phase by successive couplings of the different amino acid residues to be incorporated (from the N-terminal end to the C-terminal end in liquid phase, or from the C-terminal end to the N-terminal end in solid phase) wherein the N-terminal ends and the reactive side chains are previously blocked by conventional groups.

For solid phase synthesis, the technique described by Merrifield (1965a; 1965b) may be used in particular.

The β3 sub-unit polypeptides of the invention and their peptide fragments of interest can be used for the preparation of antibodies.

Polyclonal antibodies may be prepared by immunization of a mammal, especially a mouse or a rabbit, with a polypeptide according to the invention that is combined with an adjuvant of immunity, and then by purifying the

specific antibodies contained in the serum of the immunized animal on an affinity chromatography column on which has previously been immobilized the polypeptide that has been used as the antigen.

Monoclonal antibodies may be prepared from hybridomas according to the technique described by Kohler and Milstein (1975).

The present invention also deals with antibodies produced by the trioma technique and by the human B-cell hybridoma technique, such as described by Kozbor et al. (1983).

Antibodies of the invention also include chimeric single chain Fv antibody fragments (US Patent N° 4,946,778; Martineau et al., (1998), antibody fragments obtained through phage display libraries Ridder et al. (1995) and humanized antibodies (Leger et al., (1997)).

Antibody preparations obtained according to either protocols are useful in quantitative immuno assays for determining the presence of antigen bearing substances in biological samples. The antibodies may also be used in therapeutic compositions aimed to inhibit the biological activity of a β3 sub-unit from a voltage-gated sodium channel.

Consequently, the invention is also directed to a method for specifically detecting the presence of a β3 sub-unit from a voltage-gated channel in a sample, said method comprising the following steps of :

(a) bringing into contact a sample to be assayed with an antibody directed against a β3 sub-unit protein or to a peptide fragment thereof;

(b) detecting the antigen-antibody complex formed.

The invention also concerns a kit for detecting *in vitro* the presence of a β3 sub-unit polypeptide or a fragment thereof in a sample, wherein said kit comprises an antibody directed against a β3 sub-unit polypeptide or a peptide fragment thereof.

In a preferred embodiment, the kit further comprises a reagent allowing the detection of the antigen-antibody complexes formed, said reagent carrying optionally a label, or being able to be recognized itself by a labelled reagent, particularly in the case when the above mentioned antibody is not itself labelled.

The antibodies of the present invention are also useful as therapeutic agents capable of blocking the biological activity of brain voltage-gated sodium channel.

Thus, another object of the invention consists of a composition containing an antibody as defined herein, in combination with one or several physiologically acceptable carriers, such as those well known from the one skilled in the art.

The present invention also concerns a method for screening substances or molecules that are able to modulate the biological activity of a voltage-gated sodium channel containing a β3 sub-unit of the invention. Such a method comprises the following steps:

(a) obtaining a recombinant host cell co-expressing a β3 sub-unit and a α sub-unit, preferably an α2 sub-unit of a voltage-gated sodium channel;

(b) bringing into contact said recombinant host cell with the substance or molecule to be tested;

(c) measuring an electrical parameter within the recombinant host cell brought into contact with the substance or molecule to be tested through a voltage clamp technique or measurement of membrane potential by voltage sensitive fluorescent dyes.

A first preferred electrical parameter to be measured is the inactivation potential.

A second preferred electrical parameter to be measured is the inactivation time.

A third preferred electrical parameter to be measured is the rate of recovery of the sodium channel.

Measurement of membrane potential can be carried out using one of the techniques described in the following references: Biophys-J. 1989 Dec; 56(6): 1053-69, Biochemistry 1989 May 30; 28(11): 4536-9, Chem-Biol. 1997 Apr; 4(4): 269-77, Biophys-J. 1995 Oct; 69(4): 1272-80. All these publication are incorporated herein by reference.

In a first preferred embodiment of the above method, substances or molecules of interest are selected among those which induce changes in the activation potential, the inactivation time, or the rate of recovery of the sodium channel.

Preferred molecules or substances are those inducing a decrease in the inactivation potential, and/or a decrease in the rate of inactivation, and/or which decreases the rate of recovery from inactivation, as compared with the

same measures performed in the absence of the substance of molecule to be tested.

Molecules that may be assayed according to the method described above comprise, but are not limited to, voltage-dependent channel blockers, tetrodotoxin, lidocaine, phenytoin, carbamazepine, lamotrigine, zonisamide, riluzole, lifarizine, ralitoline, flunarizine, verapamil and carvedilol.

Other substances that may be assayed according to the method described above are molecules from the phenylacetamide familly, 6-Iodoamiloride.

Sodium channel openers may also represent good condidate molecules, such as for example carsatrin or BDF-9148 (Beiersdorf).

Therapeuticals molecules active on neuropathic pain or migrain may also be used, such as CNS-5161 (Cambridge Neuroscience's).

The invention also concerns a kit for screening substances or molecules capable of modulating the biological activity of voltage-gated sodium channel containing a β3 sub-unit, wherein said kit comprises a recombinant host cell co-expressing a β3 sub-unit and an α sub-unit, preferably an α2 sub-unit.

EXAMPLES

EXAMPLE 1: Isolation and cloning of the cDNA encoding the sodium channel β3 sub-unit from rat.

A variant of the rat pheochromocytoma cell line PC12 that has lost many of its neuroendocrine properties has been studied. Subtractive cloning to isolate cDNAs corresponding to mRNA expressed in normal PC12 cells but missing from the variant were isolated whilst identifying novel neuroendocrine-specific transcripts.

Total RNA was prepared from wild-type PC12 and variant cell lines as described by Chomcznski and Saatchi (Chomczynski and Saachi, 1987). Poly A(+) RNA was purified from the total RNA by Oligo dT Cellulose column chromatography (Pharmacia UK) (Aviv and Lader 1972). The yield of mRNA from each cell line was calculated spectrophotometrically before proceeding with subtractive hybridisation using the technique of PCR select (Clonetech, USA; Diatchenko et al. 1996). Amplified cDNA fragments derived from genes

differentially expressed in the wild-type cells were subcloned into the pTAdv plasmid (Clonetech) (Mead et al. 1994) and transformed into *E.coli* strain XLI blue (Bullock et al. 1987) to create a cDNA fragment library. Plasmid minipreps from randomly picked subclones were subjected to automated DNA sequencing and screened through DNA data base searches.

Full length coding sequence of rat β3 was isolated by screening a rat brain cDNA library with a partial clone isolated by PCR select. The rat brain cDNA library in lambdaZap (Short et al. 1988) was plated on *E. coli* strain c600hfl (Huynh et al. 1985) and phage plaques were screened with a 400 bp ^{32}P-labelled XbaI-SacI. cDNA fragment derived from the PCR select clone. Out of approximately 250,000 plaques, a single positive phage clone was isolated by plasmid rescue in pBluescript km288 plasmid using the *E.coli* XPORT, XLOLR system. (Alting-Mees & Short 1994). Both strands of the cDNA insert in km288 were subjected to automated DNA sequencing on both strands using M13 primers and internal sequence-specific primers. The resulting sense nucleic acid sequence is herein referred to as SEQ ID N°1.

EXAMPLE 2: Isolation and cloning of the cDNA encoding the sodium channel β3 sub-unit from human.

The human homologue of the novel rat β3 sub-unit was cloned from a human striatal Lambda ZAP II cDNA library obtained from Stratagene. The entire nucleotide sequence encoding the rat β3 open reading frame was amplified by PCR. This was performed using 20 mer oligonucleotides:

SEQ ID N°4
5'-ATGCCTGCCTTCAACAGATTGC-3'
(362-383 bp of the rat β3 sequence) as the forward primer, and
SEQ ID N°5
5'-TTATTCCTCCACAGGTACCA-3'
(1007-1026 bp of the rat β3 sequence) as the reverse primer.

The double stranded PCR product produced was radiolabelled by nick translation with [$\alpha^{32}P$] dATP and [$\alpha^{32}P$] d CTP and used to probe 10^6 primary plaques bound to nitrocellulose filters in a standard hybridization buffer containing 25% formamide. Single plaques giving rise to positive hybridizations were isolated and insert cDNA sequenced on an ABI 310 DNA

analyser. The resulting sense nucleic acid sequence is referred to as SEQ ID N°2.

<u>EXAMPLE 3: In situ hybridization studies of distribution f β3 sub-unit.</u>

Whole brains were dissected from adult (150-200 g) Wistar rats and snap frozen on dry ice. Brains were cut on a cryostat at 10□m, sections were thaw mounted onto poly-l-lysine coated slides, fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), dehydrated and stored under ethanol until hybridisation. The sequence and location used for analysis were as follows: rat β_1 (nucleotides 1296-1252) 5' GCTTGATGGGGTGAAGAGGGGTCGGGACAGGGACAGTAGTGGGC 3', (SEQ ID6) rat β_{13} (nucleotides389-345) 5' GGGGAAGCAATCTGTTGAAGGCAGGCATCTTTTCCACCGTAAGCG 3', (SEQ ID3) rat IIA α (nucleotides 1659-1615) 5' GCAGAATCCAGAGACTTCAGCGGGGCAGGCGGGATAGGTGTTTTC 3' (SEQ ID7). Oligonucleotides were 3' end-labelled with [^{35}S]dATP (Amersham, Bucks., U.K., 1000 Ci/mmol) by terminal deoxenucleotidyl transferase (Boehringer Mannheim, Germany) and used for hybridisation at a concentration of 400 000 cpm/100 ul of hybridisation buffer. Slides were air dried and hybridised overnight at 42°C in 150 □l buffer containing 50% formamide, 10% dextran sulfate, 50 mM DTT, 1X Denhardt's solution, 05 mg/ml denatured salmon sperm DNA and 0.5 mg/ml polyadenylic acid (all Sigma, Poole, U.K.). Sections were washed in 1×SSC at 55°C for 30 minutes, rinsed in 1×SSC, 0.1×SSC dehydrated and apposed to Kodak Biomax™ MR X-ray film (Amersham) for 10 days. For cellular resolution selected slides were susequently dipped in photographic emulsion LM-1 (Amersham), incubated for 4 weeks, developed and counter stained Cresyl Violet.

Sections were observed and photographed using a Polyvar microscope (Reichert-Jung) with camera attached.

The sequence depicted in SEQ ID N°3 is the antisense radiolabelled oligonucleotide probe used in the in situ hybridization experiments, unique to target sequence as confirmed by FASTA search (NCBI). This allows the distribution as shown in figure 1 to be determined and changes in distribution to be detected.

EXAMPLE 4: Functional expression of β3 sub-unit in a recombinant system.

Capped cRNA for rat brain type IIA α sub-unit and rat β3 sub-unit were transcribed *in vitro* from transcribed cDNAs (Promega, Southampton, UK). pBSK β3 was linearized with NotI and transcribed with T7 polymerase, while ZEMRVSP6-2580 α2 was linearized with ClaI and transcribed with SP6 polynerase. Xenopus laevis were anaesthetised by immersion in 0,3% (w/v) 3-amino benzoic acid (Sigma, Poole, U K) and Ovarian lobes were removed. Oocytes were dissociated using 0.3% (w/v) collagenase (Sigma, Poole, U K) in Ca^{2+} -free solution (82.5 mM NaCl, 2,5 mM KCl, 1 mM $MgCl_2$, 5 mM, Hepes, pH 7.6). Prepared oocytes were microinjected with 50 nl of cRNAs dissolved in water. Oocytes were incubated at 18°C in ND96 (96 mM NaCl; 2mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, pH 7.6). Two-electrode voltage clamp recordings were performed 3-6 days after microinjection of cRNAs using a Gene Clamp 500 amplifier (Axon Instruments, CA, USA) interfaced to a Digidata 1200 A/D board with Clampex software (v6, Axon Instruments, CA, USA). Oocytes were continually perfused with ND96, Microelectrodes filled with 3 M KCl had resistances between 0.5-2 MΩ. Currents were sampled at 10 kHz and filtered at 2 kHz. Data were analyzed using Clampfit (v6, Axon Instruments, CA, USA) and Prism (v2, Graphpad Software, CA, USA).

Inward Na^+ currents were induced by applying 5 mV depolarizing pulses from a holding potential of -100 mV, from -80 mV to + 30 mV. Na^+ currents

recorded from oocytes expressing IIA α subunit alone. Inactivation at –10 mV was best-fitted with a double exponential function, where $\tau_1 = 2 \pm 0.3$ ms and $\tau_2 = 12.7 \pm 2.4$ ms ($n = 4$). **b.** Na^+ currents recorded from oocytes coexpressing IIA α and β_1 subunits. Inactivation was best-fitted with a double exponential function, where $\tau_1 = 1.3 \pm 0.3$ ms and $\tau_2 = 22.7 \pm 7.7$ ms at -10 mV ($n = 4$). **c.** Na^+ currents recorded from oocytes coexpressing IIA α and the β_3 subunits. Inactivation was best-fitted with a double exponential function, where $\tau_1 = 1 \pm 0.1$ ms and $\tau_2 = 23.8 \pm 6.3$ ms at -10 mV ($n = 4$).

Comparison of steady-state inactivation curves for Na^+ currents recorded from oocytes coexpressing $\alpha 2$ and $\beta 3$ with oocytes expressing }$\alpha 2$ alone. Data were fitted to Boltzmann function, $g/\ g_{max}$ = J/ {1 + exp[(V-$V_{1/2}$)/k]}, where $V_{1/2}$ is the midpoint and k is the slope factor. For $\alpha 2 + \beta 3$, $V_{1/2}$= - 49.4 mV, k = 10.1 mV, while for $\alpha 2$, $V_{1/2}$ = -41.3 mV, k = 9.1 mV. Co-expression of $\beta 3$ with $\alpha 2$ causes a hyperpolarizing shift in the steady-state inactivation curve.

Comparison of rate of recovery from inactivation for Na^+ currents recorded from oocytes co-expressing $\alpha 2$ and $\beta 3$ with oocytes expressing $\alpha 2$ alone. Data were fitted with double exponential functions. For $\alpha 2 + \beta 3$, $\tau 1$ = 1.9 ms, $\tau 2$ = 198 ms while for $\alpha 2$, $\tau 1$ = 3.8 ms, $\tau 2$= 264 ms. Co-expression of $\beta 3$ with $\alpha 2$ increases the rate of recovery from inactivation.

The same procedures were used to express the human form of $\beta 3$ and to measure the effects of co-expression with the type IIA α. For type IIA α alone Inactivation was best-fitted with a double exponential function, where $\tau_1 = 1.78 \pm 0.4$ ms and $\tau_2 = 13 \pm 1.25$ ($n = 4$). For type IIA α and human $\beta 3$, inactivation was best-fitted with a double exponential function, where $\tau_1 = 1 \pm 0.1$ ms and $\tau_2 = 9.1 \pm 1.4$ ms. t1/(t1+t2)= 0.72+0.03.

This methodology is a technology for detecting changes in the function of the sodium channel complex as shown in figure 2.

REFERENCES:

• Alting-Mees MA and Short J.M. 1994, Strategies 7 70-72 (*E. coli* XPORT/XLOLR helper phage RE704 system).

• Avid H. and Leder P., (1972) Proc. Natl. Acad. Sci., 69:1408-1412.

• Beaucage et al., Tetrahedron Lett (1981) 22: 1859-1862.

• Bullock WO et al., 1987 Biotechniques 5; 376-379: (*E. coli* XLI Blue).

• Brown EI., Belagaje R, Ryan MJ, Khorana HG, Methods Enzymol (1979); 68: 109-151.

• Catterall, W.A. Yeasty Brew yields novel calcium channel inhibitor Nature Biotec 16 1998 906.

• Chen et al., (1987) Mol. Cell. Biol., 7:2745-2752.

• Chomczynski P and Saachi N. 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem 162; 156-159.

• Diatchenko L. et al., 1996 . PNAS 93: 6025-6030.

• Fraley et al. (1979), Proc. Natl. Acad. Sci. USA , 76:3348-3352.

• Feldman and Steg, (1996) Medecine/Sciences, synthese, 12:47:55.

• Flotte et al., (1992) Am. J. Respir. Cell Mol. Biol., 7:349:356.

• Fields and Song, (1989) Nature, 340:245-246.

• Graham et al., (1973) Virology, 52: 456-457.

• Green et al., Ann. Rev. Biochem. 55:569-597 (1986).

• Gopal (1985), Mol. Cell. Biol., 5:1188-1190.

• Hames. B.D. Higgins, S.J. (1993). Transcription and translation (Practical approach series, ISBN 100190) Imprint Oxford: IRL Press, 1993.

• Harland et al., (1985) J. Cell. Biol. , 101: 1094-1095.

• Huynh TV, Young RA, Davis RW. In DNA cloning (DM Glover, ed). Vol. 1, pp 56-110. IRL Press, Oxford, England 1985 (*E. coli* C600hfl).

• Huygen et al., (1996) Nature Medicine, 2(8):893-898.

• Houbenweyl, (1974), in Meuthode der Organischen Chemie, E. Wunsch Ed., Volume 15-I et 15-II, Thieme, Stuttgart.

• Izant JG, Weintraub G, Cell 1984 Apr; 36(4):1007-15.

•James, W. & Agnew, W. (1989). Alpha-(2-8)-polysialic acid immunoreactivity in voltage-sensitive sodium channel of eel electric organ. Proceedings of the Royal Society B 237, 233-245.

• Julan et al., (1992) J. Gen. Virol., 73: 3251-3255.

- Koch Y. (1977), Biochem. Biophys. Res. Commun., 74: 488-491.
- Kohler G. and Milstein C., (1975) Nature, 256:495.
- Kozbor et al., (1983) Hybridoma, 2(1):7-16.
- Leger OJ, et al. (1997) Hum Antibodies , 8(1):3-16.
- Makielski; J. Limberis, J., Chang S., Fan, Z & Kyle, J. (1996). Molecular Pharmcology 49, 30-39.
- Martineau P, Jones P, Winter G. (1998), J. Mol Biol, 280(1):117-127.
- Merrifield RB, 1965a, Nature, 207(996):522-523.
- Merrifield RB, 1965b, Nature, 207 (996):522-523.
- McLaughlin BA et al., (1996), Am. J. Hum. Genet, 59:561-569.
- Nada S. et al., (1993), Cell. 73:1125-1135.
- Narang SA, Hsiung HM, Brousseau R, Methods Enzymol 1979: 68: 90-98.
- Mead DA et al. Bio/Technology 9:657-633:1994 (pT-ADV plasmid).
- Nicolau C. et al., (1987), Methods Enzymol., 149:157-76.
- Ohno et al., (1994), Science, 265:781-784.
- O'Reilly et al., (1992) Baculovirus expression vectors: a Laboratory Manual. W.H. Freeman and Co., New York.
- Patton, D., West. J. Catterall, W. & Goldin, A. (1992). Proceedings of the National Academy of Sciences of the USA 89, 10905-10909.
- Potter et al., (1984) Proc Natl Acad Sci U S A : 81-22):7161-5.
- Qu, Y., Isom, L., Westenbroek, R. Rogers, J. Tanada, T., McCormick, K., Scheuer, T. & Catterall, W./ (1995). Modulation of cardia Na^{+} channel expression in Xenopus oocytes by beta 1-sub-units. Journal of Biological Chemistry 270, 25696-25701.
- Tur-Kaspa et al., (1986), Mol. Cell. Biol., 6:716-718.
- Tacson et al., (1996) Nature Medicine, 2(8):888-892.
- Rossi et al. Pharmacol. Ther. 50:245-254 (1991).
- Roth J.A. et al., (1996) Nature Medicine, 2(9):985-991.
- Ridder R. Schmitz R, Legay F, Gram H, (1995) Biotechnology (NY), 13(3):255-260.
- Roux et al., (1989) Proc. Natl Acad. Sci. USA, 86:9079-9083.
- Russell DW. Hirata RK 1998. Human gene targeting by viral vectors Nature Genetics. 18(4):325-330.
- Rougeot, c. et al., (1994), Eur. J. Biochem., 219(3):765-773.
- Samulski et al., (1989) J. Virol. 63: 3822-3828.

- Smith et al., (1983), Mol. Cell. Biol., 3:2156-2165.
- Sternberg N.L. (1992), Trends Genet, 8: 1-16.
- Sternberg N.L. (1994) Mamm. Genome, 5:397-404.
- Short JM, Fernandez JM, Sarge JA, Huse WD 1988. NAR 16-7583-7600.
- Shena et al., Science 270: 467-470, 1995.
- Shena et al., 1996 Proc Natl Acad Sci U S A. 93(20):10614-10619.
- Sambrook, J. Fritsch, E.F. and T. Maniatis (1989). Molecular cloning: a laboratory manual, 2ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Sanchez-Pescador R., (1988), J. Clin. Microbiol., 26(10): 1934-1938.
- Sczakiel G. et al., (1995) Trends Microbiol., 3(6): 213-217.
- Tacson et al., (1996) Nature Medicine, 2(8):888-1190
- Urdea et al., MS (1988) Nucleic Acids Research, 11: 4937-4957.
- Urdea et al., MS (1991) Nucleic Acids Symp Ser., 24: 197-200.
- Young, K. et al. Nature Biotech 16 1998 946-950.
- Zhang Z. & Galilco, D.S. (1998). Neuroscience 18:6928-6938.

Claims:

1. A purified or isolated nucleic acid encoding a β3 sub-unit from a voltage-gated sodium channel, or a sequence complementary thereto.

2. The nucleic acid of claim 1, which encodes a β3 sub-unit from th voltage-gated sodium channel present in the rat brain, or a sequence complementary thereto.

3. The nucleic acid of claim 1, which encodes the β3 sub-unit from the voltage-gated sodium channel present in the human brain, or a sequence complementary thereto.

4. A purified or isolated nucleic acid encoding a polypeptide having at least 80% amino acid identity with the β3 sub-unit polypeptide of the amino acid sequence of SEQ ID NO 8, or with a peptide fragment thereof, or a sequence complementary thereto.

5. A purified or isolated nucleic acid encoding a polypeptide having at least 80% amino acid identity with the β3 sub-unit polypeptide of the amino acid sequence of SEQ ID NO 9, or a sequence complementary thereto.

6. A purified or isolated nucleic acid having at least 90% nucleotide identity with the nucleotide sequence of SEQ ID NO 1, or a sequence complementary thereto.

7. A purified or isolated nucleic acid comprising a polynucleotide having at least 90% nucleotide identity with the sequence beginning at the nucleotide located in position 363 and ending at the nucleotide located in position 1010 of the nucleotide sequence of SEQ ID N°1.

8. A purified or isolated nucleic acid comprising a sequence beginning at the nucleotide located in position 1 and ending at the nucleotide located in position 362 of the nucleotide sequence of SEQ ID N°1.

9. A purified or isolated nucleic acid comprising a sequence beginning at the nucleotide located in position 1011 and ending at the nucleotide located in position 2220 of the nucleotide sequence of SEQ ID N°1.

10. A purified or isolated nucleic acid having at least 90% nucleotide identity with the nucleotide sequence of SEQ ID NO 2, or a sequence complementary thereto.

11. A purified or isolated nucleic acid comprising a polynucleotide having at least 90% nucleotide identity with the sequence beginning at the

nucleotide located in position 376 and ending at the nucleotide in position 1023 of the nucleotide sequence of SEQ ID N°2.

12. A purified or isolated nucleic acid comprising a sequence beginning at the nucleotide located in position 1 and ending at the nucleotide located in position 375 of the nucleotide sequence of SEQ ID N°2.

13. A purified or isolated nucleic acid comprising a sequence beginning at the nucleotide located in position 1024 and ending at the nucleotide located in position 1261 of the nucleotide sequence of SEQ ID N°2.

14. A purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid according to any one of claims 1 to 6 and 10.

15. A purified or isolated nucleic acid comprising at least 10 consecutive nucleotides of the nucleotide sequence of SEQ ID NO 1, or a sequence complementary thereto.

16. A purified or isolated nucleic acid comprising at least 10 consecutive nucleotides of the nucleotide sequence of SEQ ID NO 2, or a sequence complementary thereto.

17. A purified or isolated nucleic acid according to any one of claims 15 or 16, which is an antisense nucleic acid.

18. The nucleic acid of claim 17 which consists of the nucleotide sequence of SEQ ID N°3.

19. A polynucleotide probe hybridizing, under stringent hybridization conditions, with a nucleic acid according to any one of claims 1 to 16.

20. The polynucleotide probe of claim 17, which is labelled by a detectable molecule.

21. A polynucleotide primer hybridizing, under stringent hybridization conditions, with a nucleic acid according to any one of claims 1 to 16.

22. A polynucleotide primer according to claim 21, wherein said primer comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs 4 and 5.

23. A method for the amplification of a β3 subunit nucleic acid, said method comprising the steps of :

a) contacting a test sample suspected of containing the targeted β3 subunit nucleic acid or a fragment thereof with amplification reaction

reagents comprising a pair of amplification primers which can hybridize to either side of a β3 subunit nucleic acid region of a nucleic acid according to any one claims 1 to 16, and

b) optionally, detecting the amplification products.

24. The method according to claim 23, wherein the amplification primers are respectively the nucleotide sequences of SEQ ID Nos 4 and 5.

25. A kit for the amplification of a β3 subunit nucleotide sequence, wherein said kit comprises :

a) a pair of amplification primers which can hybridize to a β3 subunit nucleic acid according to any one of claims 1 to 16, and

b) optionally, the reagents necessary for performing the amplification reaction.

26. The kit of claim 25, wherein the amplification primers are respectively the nucleotide sequences of SEQ ID Nos 4 and 5.

27. A method for detecting the presence of polynucleotide comprising a nucleic acid according to any one of claims 1 to 16 in a sample, wherein said method comprises the steps of :

a) bringing into contact a nucleic acid probe or a plurality of nucleic acid probes which can hybridize, under stringent hybridization conditions, to a nucleotide sequence included in a nucleic acid according to any one of claims 1 to 16, and the sample to be assayed;

b) detecting the hybrid complex formed between the probe or the plurality of probes and the nucleic acid in the sample.

28. The method of claim 27, wherein the nucleic acid probe or the plurality of nucleic acid probes are immobilized on a substrate.

29. The method of claim 27, wherein the nucleic acid probe or the plurality of nucleic acid probes is labeled with a detectable molecule.

30. A kit for detecting the presence of a polynucleotide comprising a nucleic acid according to any one of claims 1 to 16, wherein said kit comprises :

a) a nucleic acid probe or a plurality of nucleic acid probes which can hybridize, under stringent hybridization conditions, to a nucleotide sequence included in a nucleic acid according to any one of claims 1 to 16;

b) optionally, the reagents necessary to perform the hybridization reaction.

31. The kit of claim 30, wherein the nucleic acid probe or the plurality of nucleic acid probes are immobilized on a substrate.

32. The kit of claim 30, wherein the nucleic acid probe or the plurality of nucleic acid probes are labeled with a detectable molecule.

33. A recombinant vector comprising a nucleic acid according to any one of claims 1 to 18.

34. A recombinant host cell comprising a nucleic acid according to any one of claims 1 to 18 or a recombinant vector according to claim 33.

35. A method for producing a polypeptide encoded by a nucleic acid according to any one of claims 1 to 7, 10, 11 and, 14 to16, wherein said method comprises the following steps of :

a) culturing, in an appropriate culture medium, a host cell previously transformed or transfected with a polynucleotide according to any one of claims 1 to 7, 10, 11 and, 14 to 16, or with a recombinant vector according to claim 33;

b) harvesting the culture medium thus conditioned or lyse the host cell, for example by sonication or by osmotic shock;

c) separating or purifying, from said culture medium, or from the pellet of the resulting cell lysate, the thus produced polypeptide of interest;

36. The method of claim 35, wherein before step a) the polynucleotide according to any one of claims 1 to 7, 10, 11 and 14 to16 has previously undergone an amplification reaction.

37. The method of claim 36, wherein after step c), the polypeptide of interest thus produced is characterized.

38. A purified or isolated polypeptide comprising the amino acid sequence of the β3 sub-unit from a voltage-gated sodium channel, or a peptide fragment thereof.

39. The polypeptide of claim 38, which comprises the amino acid sequence of the β3 sub-unit from a voltage-gated sodium channel present in the rat brain, or a peptide fragment thereof.

40. The polypeptide of claim 38, which comprises the amino acid sequence of the β3 sub-unit from a voltage-gated sodium channel present in the human brain, or a peptide fragment thereof.

41. A purified or isolated polypeptide comprising an amino acid sequence having at least 90% amino acid identity with the amino acid sequence of SEQ ID NO 8, or a peptide fragment thereof.

42. A purified or isolated polypeptide comprising an amino acid sequence having at least 90% amino acid identity with the amino acid sequence of SEQ ID NO 9, or a peptide fragment thereof.

43. A purified or isolated polypeptide encoded by a nucleic acid of any one of claims 1 to 7, 10, 11, 14 to 16.

44. An antibody directed against a polypeptide according to any one of claims 38 to 43.

45. The antibody of claim 44 which is detectably labeled.

46. A method for detecting the presence of a polypeptide according to any one of claims 38 to 42 in a sample, wherein said method comprises the following steps of :

a) bringing into contact a sample to be assayed with an antibody directed against a polypeptide according to any one of claims 38 to 43;

b) detecting the antigen-antibody complex formed.

47. A kit for detecting *in vitro* the presence of a polypeptide according to any one of claims 38 to 43 in a sample, wherein said kit comprises :

a) an antibody directed against a polypeptide according to any one of claims 38 to 43;

b) optionally, a reagent allowing the detection of the antigen-antibody complex formed.

48. A composition containing a nucleic acid according to anyone of claims 1-18 in combination with one or several physiologically acceptable carriers.

49. A composition containing an antibody according to claim 44, in combination with one or several physiologically acceptable carriers.

ABSTRACT OF DISCLOSURE

A novel family of beta sub-unit proteins from a voltage-gated sodium channel, nucleic acids encoding them and therapeutic or diagnostic uses thereof.

The present invention relates to a novel family of beta sub-unit proteins from a voltage-gated sodium channel, and particularly the human and the rat beta sub-units which have been collectively identified as β3, in view of their close structural relationship.

The invention also deals with the use of the β3 sub-unit polypeptide or a fragment thereof as well as of the nucleic acids encoding same for therapeutic and diagnostic purposes.

Figure 1

```
human beta 3 ORF     (1) ATGCCTGCCTTCAATAGATTGTTTCCCCTGGCTTCTCTCGTGCTTATCTA
  Rat Beta 3 ORF     (1) ATGCCTGCCTTCAACAGATTGCTTCCCCTAGCTTCTCTAGTGCTCATCTA
       Consensus     (1) ATGCCTGCCTTCAA AGATTG TTCCCCT GCTTCTCT GTGCT ATCTA
                         51                                             100
human beta 3 ORF    (51) CTGGGTCAGTGTCTGCTTCCCTGTGTGTGTGGAAGTGCCCTCGGAGACGG
  Rat Beta 3 ORF    (51) CTGGGTCAGAGTCTGCTTCCCTGTGTGTGTGGAAGTGCCCTCGGAGACAG
       Consensus    (51) CTGGGTCAG GTCTGCTTCCCTGTGTGTGTGGAAGTGCCCTCGGAGAC G
                         101                                            150
human beta 3 ORF   (101) AGGCCGTGCAGGGCAACCCCATGAAGCTGCGCTGCATCTCCTGCATGAAG
  Rat Beta 3 ORF   (101) AAGCGGTGCAGGGCAATCCCATGAAGCTGAGGTGCATCTCCTGCATGAAG
       Consensus   (101) A GC GTGCAGGGCAA CCCATGAAGCTG G TGCATCTCCTGCATGAAG
                         151                                            200
human beta 3 ORF   (151) AGAGAGGAGGTGGAGGCCACCACGGTGGTGGAATGGTTCTACAGGCCCGA
  Rat Beta 3 ORF   (151) AGGGAGGAGGTGGAGGCCACCACTGTGGTGGAGTGGTTCTACAGGCCTGA
       Consensus   (151) AG GAGGAGGTGGAGGCCACCAC GTGGTGGA TGGTTCTACAGGCC GA
                         201                                            250
human beta 3 ORF   (201) GGGCGGTAAAGATTTCCTTATTTACGAGTATCGGAATGGCCACCAGGAGG
  Rat Beta 3 ORF   (201) GGGCGGTAAAGATTTCCTTATATATGAGTATCGGAATGGCCACCAGGAAG
       Consensus   (201) GGGCGGTAAAGATTTCCTTAT TA GAGTATCGGAATGGCCACCAGGA G
                         251                                            300
human beta 3 ORF   (251) TGGAGAGCCCCTTTCAGGGGCGCCTGCAGTGGAATGGCAGCAAGGACCTG
  Rat Beta 3 ORF   (251) TGGAGAGCCCCTTCCAAGGCCGTCTGCAGTGGAATGGGAGCAAAGACCTG
       Consensus   (251) TGGAGAGCCCCTT CA GG CG CTGCAGTGGAATGG AGCAA GACCTG
                         301                                            350
human beta 3 ORF   (301) CAGGACGTGTCCATCACTGTGCTCAACGTCACTCTGAACGACTCTGGCCT
  Rat Beta 3 ORF   (301) CAGGACGTATCCATCACTGTACTCAATGTCACTTTGAATGACTCTGGCCT
       Consensus   (301) CAGGACGT TCCATCACTGT CTCAA GTCACT TGAA GACTCTGGCCT
                         351                                            400
human beta 3 ORF   (351) CTACACCTGCAATGTGTCCCGGGAGTTTGAGTTTGAGGCGCATCGGCCCT
  Rat Beta 3 ORF   (351) CTACACATGCAATGTGTCCAGGGAGTTCGAATTCGAGGCACACAGGCCTT
       Consensus   (351) CTACAC TGCAATGTGTCC GGGAGTT GA TT GAGGC CA  GGCC T
                         401                                            450
human beta 3 ORF   (401) TTGTGAAGACGACGCGGCTGATCCCCCTAAGAGTCACCGAGGAGGCTGGA
  Rat Beta 3 ORF   (401) TTGTGAAGACCACGAGACTGATACCTTTGCGAGTCACTGAAGAGGCGGGA
       Consensus   (401) TTGTGAAGAC ACG G CTGAT CC   T  GAGTCAC GA GAGGC GGA
                         451                                            500
human beta 3 ORF   (451) GAGGACTTCACCTCTGTGGTCTCAGAAATCATGATGTACATCCTTCTGGT
  Rat Beta 3 ORF   (451) GAAGACTTCACCTCCGTGGTCTCGGAAATCATGATGTACATCCTCCTGGT
       Consensus   (451) GA GACTTCACCTC GTGGTCTC GAAATCATGATGTACATCCT CTGGT
                         501                                            550
human beta 3 ORF   (501) CTTCCTCACCCTGTGGCTGCTCATCGAGATGATATATTGCTACAGAAAGG
  Rat Beta 3 ORF   (501) CTTCCTCACCTTGTGGCTGTTTATTGAGATGATCTATTGCTACAGAAAGG
       Consensus   (501) CTTCCTCACC TGTGGCTG T AT GAGATGAT TATTGCTACAGAAAGG
                         551                                            600
human beta 3 ORF   (551) TCTCAAAAGCCGAAGAGGCAGCCCAAGAAAACGCGTCTGACTACCTTGCC
  Rat Beta 3 ORF   (551) TCTCTAAGGCCGAAGAGGCAGCACAGGAAAATGCGTCTGACTACCTTGCT
       Consensus   (551) TCTC AA GCCGAAGAGGCAGC CA GAAAA GCGTCTGACTACCTTGC
                         601                                         648
human beta 3 ORF   (601) ATCCCATCTGAGAACAAGGAGAACTCTGCGGTACCAGTGGAGGAATAG
  Rat Beta 3 ORF   (601) ATCCCTTCAGAGAACAAGGAGAACTCTGTGGTACCTGTGGAGGAATAA
       Consensus   (601) ATCCC TC GAGAACAAGGAGAACTCTG GGTACC GTGGAGGAATA
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SEQUENCE LISTING

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<120> A novel family of beta sub-unit proteins from a voltage-gated sodium channel, nucleic acids encoding them and therapeutic or diagnostic uses thereof

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Met Lys Arg Glu Glu Val Glu Ala Thr Thr Val Val Glu Trp Phe Tyr
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Arg Pro Glu Gly Gly Lys Asp Phe Leu Ile Tyr Glu Tyr Arg Asn Gly
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His Gln Glu Val Glu Ser Pro Phe Gln Gly Arg Leu Gln Trp Asn Gly
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Ser Lys Asp Leu Gln Asp Val Ser Ile Thr Val Leu Asn Val Thr Leu
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Asn Asp Ser Gly Leu Tyr Thr Cys Asn Val Ser Arg Glu Phe Glu Phe
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Met Ile Tyr Cys Tyr Arg Lys Val Ser Lys Ala Glu Glu Ala Ala Gln
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Thr Glu Ala Val Gln Gly Asn Pro Met Lys Leu Arg Cys Ile Ser Cys
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Met Lys Arg Glu Glu Val Glu Ala Thr Thr Val Val Glu Trp Phe Tyr
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Arg Pro Glu Gly Gly Lys Asp Phe Leu Ile Tyr Glu Tyr Arg Asn Gly
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His Gln Glu Val Glu Ser Pro Phe Gln Gly Arg Leu Gln Trp Asn Gly
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Ser Lys Asp Leu Gln Asp Val Ser Ile Thr Val Leu Asn Val Thr Leu
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Asn Asp Ser Gly Leu Tyr Thr Cys Asn Val Ser Arg Glu Phe Glu Phe
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Glu Ala His Arg Pro Phe Val Lys Thr Thr Arg Leu Ile Pro Leu Arg
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Val Thr Glu Glu Ala Gly Glu Asp Phe Thr Ser Val Val Ser Glu Ile
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Met Met Tyr Ile Leu Leu Val Phe Leu Thr Leu Trp Leu Leu Ile Glu
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Figure 2

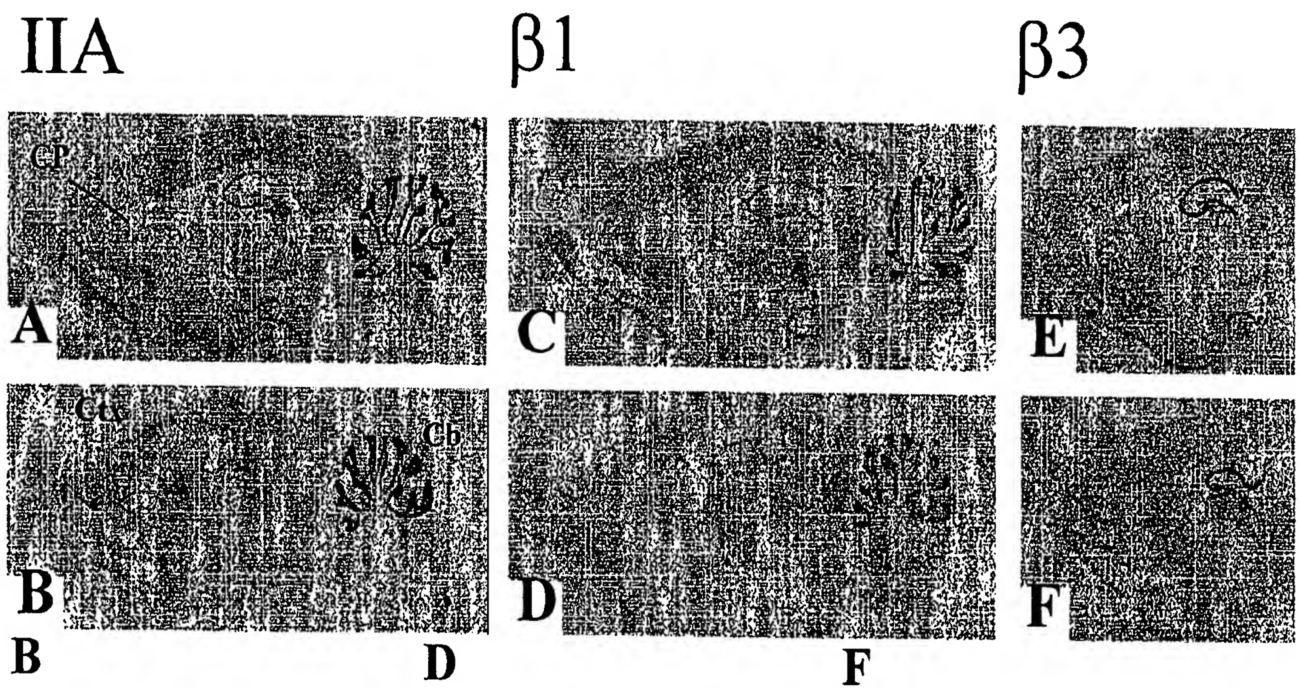

Figure 3